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SERUM REACTIONS IN INFLUENZA *

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There is apparently little information on the serology of influenza which is of certain etiologic or diagnostic importance. Cantani,[1] Vagedes,[2] Fichtner[3] and Ghedini[4] have described a few positive agglutination reactions with serum of acute and convalescent influenza cases and Pfeiffer's bacillus, but several of them, notably Fichtner, have also mentioned positive reactions, although usually in lower dilutions, with normal serum, so that the specificity of the reaction is questionable. Odaira[5] noted a tendency to spontaneous clumping of influenza bacilli in normal salt solution. The observation of Vagedes that he obtained better results when the tests were incubated at 37 C. for from 20 to 24 hours is suggestive in view of our results at a higher temperature.

We find no reference to the use of other immunity reactions in influenza cases.

Our experiments herein briefly reported are at least suggestive from the standpoint of etiology and diagnosis, and would seem to be self-controlled, owing to the groups of cases studied, although the number of normal controls is limited.

RABBIT IMMUNE SERUM

Several rabbits were immunized by from 6 to 8 injections, the majority intravenously, but a few subcutaneously, of a mixed vaccine comprised of equal parts of 10 different strains of B. influenzae. These strains were originally isolated in the laboratory of Dr. W. H. Park during Sept., 1918, and had been derived from the nasopharynx or lung of cases of influenza. They were kindly placed at our disposal by Dr. G. H. Smith, of the Yale Medical School, for the purpose of this investigation. These strains were uniformly grown on a "chocolate" medium prepared by adding 5% of defibrinated rabbit or human

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[1] Ztschr. f. Hyg. u. Infektionskr., 1903, 42, p. 505.

[2] Deutsch. militärärztl. Ztschr., 1903, 32, p. 236. Abstract in Baumgarten's Jahresbericht über die path. Mikroorganisman, 1903, 19, p. 244.

[3] Deutsche militärärztl Ztschr., 1906, 35, p. 337. Abstract in Baumgarten's Jahresbericht. 1906, 22, p. 207.

[4] Abst. in Centralbl. f. Bakt., 1 Abt., Ref. 1908, 42, p. 177.

[5] Centralbl. f. Bakteriol. I. O., 1912, 61, p. 289.

blood to 5% glycerol agar at a temperature of 95 C. On this medium all the cultures with which we have dealt grow very profusely, so that in 24 hours the Blake bottle is covered with a uniform creamy layer. Suspensions from these cultures are readily made by rocking the bottles with salt solution. They usually sediment very slowly when prepared in this manner, and do not agglutinate spontaneously with normal salt solutions, as has apparently often been the case when cultures from a plain blood medium are employed.

Our rabbits received injections beginning with 0.5 c c of a thick suspension of the mixed cultures killed by the addition of 0.5% phenol at first. On subsequent injections living cultures were employed. It was found that injections could be given at short intervals without producing any symptoms in the rabbits except slight loss in weight. The serum from these animals was tested after 6 or 8 injections.

Negative Tests.—The following reactions were tried with the serum of the immunized animals, with completely negative results:

1. Agglutination tests with dilutions of the serum of from 1:10 to 1:160 in a total volume of 1 c c, by adding three drops of a thick suspension of phenolated mixed culture as described. Several of the separate cultures used to immunize animals were also tried, and also with negative results. In these experiments the tubes were incubated at 37 C. for 2 hours and then allowed to stand at room temperature for 18 hours.

2. Pfeiffer's phenomena of bacteriolysis (Cantani).

3. The conglutination reaction (Bordet and Gay).

4. Precipitin tests with an antigen obtained by grinding the vaccine of mixed influenza strains precipitated and dried by alcohol and ether, ground, extracted with phenolated salt solution, and centrifugalizing until the supernatant fluid was perfectly clear.

Positive Reactions with Rabbit Immune Serum. Fixation Reactions.—These reactions were carried out with the immune serum and also with the serum of patients, as will be described later, with the following method: Tubes containing 0.1 c c or less of the serum to be tested with and without the antigen. The antigen employed in this experiment consisted of a mixture of the 10 influenza strains, centrifugalized, killed and precipitated with absolute alcohol and absolute ether, and dried over calcium chlorid. This dried vaccine was then ground in an agate mortar and suspended in 0.5% phenolated salt solution in the proportions of 1 mg. of dried bacteria to 1 c c; 2½ units of fresh guinea-pig serum were added to the combination of antigen and serum, and one half of the inhibiting dose of antigen with a control normal serum was employed. After incubation for one-half hour at 37 C., 0.5 c c of washed sheep blood was added, plus 2 hemolytic units of rabbits antisheep serum. The reactions were observed after one-half hour's incubation at 37 C., and on standing at room temperature until the following day.

The serum of immune rabbits was found to give a complete inhibition of hemolysis, that is, complete fixation, in a dose which in one instance was as low as 1/320 of a cubic centimeter.

Agglutination Reactions at 55 C.—The rabbit immune serum gave negative results in agglutination tests incubated at 37 C. for 2 hours, as already stated. The observations of Vagedes, and particularly a personal communication from Major Leslie H. Spooner, led us to try the effect of longer incubation at body temperature. This method gave positive results with the rabbit immune serum.

Further experiments showed that the best results are procured by incubating at 55 C., as is the case with meningococcus agglutinations. Clumping and complete sedimentation in from 3 to 6 hours was found with the serum of different immune rabbits in dilutions of from 1:100 to 1:800, while normal serum controls remain negative at 1:50. There is some evidence that these agglutinins are quickly lost, as an unheated serum of high titer tested at intervals within a month dropped from 1:800 to 1:50.

There was also a variation in the agglutinability of different strains of B. influenzae which strongly suggests the existence of different groups or varieties of influenza bacilli. Our successful results both with rabbit immune and human serum have been with one of the strains used in immunizing the rabbits (strain C from nasopharynx, Sept. 5, 1918). Two other strains of B. influenzae were also tried with the rabbit serum. The one strain (strain L, lung, Sept. 21, 1918) had not been used in the mixed vaccine with which the animals were immunized. The other strain had been derived from the nasopharynx of an acute case of influenza within 10 days. The serum failed to agglutinate these strains in a dilution of 1:50.

SERUM REACTIONS WITH INFLUENZA CASES

Serum from acute cases of influenza; from recovered cases of influenza; from persons vaccinated by means of three injections, totalling three thousand million organisms, of a mixed vaccine prepared from eighteen Park strains, and from a few normal cases have been tested.

Negative Reactions. — The following reactions gave completely negative results when tested with serum representing the categories just mentioned:

1. Agglutination reactions with short incubation at 37 C., in accordance with the usual method, as previously outlined.
2. Conglutination reactions.
3. Opsonic tests.
4. Precipitin tests.
5. Intradermal injections of alcohol-killed, ground cultures of B. influenzae in a series of vaccinated and recovered cases.
6. Fixation tests, using as antigen the sputums of influenza cases prepared after the method of Krumwiede and Valentine,[6] using both the serum of the patient from whom the sputum was derived, and of several other acute cases.
7. Fixation tests, using the urine as an antigen, prepared after the method described by Dochez and Avery[7] for the precipitin tests in pneumonia, both with the serum of the individual patient and other patients as antibodies.
8. Fixation tests were tried with two separate antigens, prepared in the manner described, from gram-negative diplococci that were frequently found in the bacterial flora of the acute cases.

Positive Serum Reactions.—Fixation reactions were obtained in 40% of twenty-five vaccinated cases, and in only one of twenty-nine acute cases of influenza. They were negative in normal individuals and in the recovered cases.

Agglutination reactions with phenolated suspensions of influenza bacilli with short incubation at 37 C. were negative, as in the case of the rabbit immune serum. Definite positive reactions were at once found on incubation at 55 C. with some of the serum, while others remained negative. In from 3 to 6 hours large mucoid floccules appear in the positive tubes which promptly settle in a compact mass at the bottom. In the negative tubes the suspension of bacteria remained homogeneous. If the tubes were removed from the 55 C. bath at the end of 6 hours and allowed to stand over night at room temperature, the readings are the same on the following day, in the majority of instances. With some of the phenolated suspensions, however, the differentiation between serum that at 6 hours are distinct may not be clear on the following day. We must confess that we have been led to frequent repetitions of these experiments through some uncertainty as to the correct criterion of differentiation. The results at 6-hour reading are sharp, and owing to the considerable number of serums from different groups of cases tested, would seem to be self-controlled. Nearly 90% of the acute cases of influenza gave positive agglutination reactions in a dilution of from 1:40 to 1:160, whereas all marked as negative failed in a dilution of 1:10. The serum of previously vaccinated individuals were positive in 45%, and recovered cases in 38.5%, apparently irrespective of the time after recovery. Very few normal serums were available. It is also unfortunate that the serums of other acute febrile conditions as a control on the positive reactions were not available.

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[6] Jour. Am. Med. Assn., 1918, 70, p. 513.

[7] Jour. Exper. Med., 1917, 26, p. 477.

TABLE 1

FIXATION REACTIONS WITH MIXED INFLUENZA ANTIGEN AND HUMAN SERUM

	No. Tested	Positive	Percentage
Acute cases of influenza	29	1	3
Recovered cases (from 10 to 48 days after normal temperature)	17	0	0
Vaccinated (from 2 to 37 days after last injection)	25	10	40
Normal serum	5	0	0

TABLE 2

AGGLUTINATION REACTIONS AT 55 C. WITH CULTURE C

	Number Tested	Positive	Percentage
Acute cases	25	22	88.0
Vaccinated	24	11	45.8
Recovered	13	5	38.5
Normal serum	9	0	0.0

It should be added that the results noted were obtained with a phenolated suspension of culture C. Reactions with culture L were uniformly negative. A few positive reactions were obtained with the serum of acute cases and culture 69, but most of them were negative, as were the other serums studied.

SUMMARY

The serum of rabbits immunized by means of a suspension of mixed cultures of B. influenzae gave for the most part negative immunity reactions. Fixation antibodies were found in high dilutions, using a preparation of the same mixed cultures as antigen. Agglutination reactions were negative when incubated for a short period at 37 C., but positive in from 3 to 6 hours at 55 C. The immune serum failed to react with two strains of B. influenzae that had not been used in immunizing, thus suggesting the existence of separate groups of B. influenzae.

The serum of acute influenza cases gave a positive agglutination reaction at 55 C. in dilutions of 1:80 and above, in the majority of instances (88%) with one of the strains of B. influenzae tested. A few tests with another recently isolated strain from our epidemic were also positive in a few instances. Another strain was uniformly negative. Fixation reactions were negative in all but one case with a mixed antigen.

The serum from individuals that had recovered from influenza gave agglutination reactions at 55 C. in about two thirds of the instances. Fixation reactions were negative.

The serum of individuals that had been vaccinated gave agglutinations at 55 C. in 45% of the cases. Fixation reactions were positive in 40%.

The normal cases, although few in number, were uniformly negative.